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THE EXCRETION OF INULIN BY THE DOG

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The present paper reports observations on the excretion of the polysaccharid inulin by the normal and phlorizinized dog, these observations having been made prior to, or concurrently with, the observations on man recently reported by Shannon and Smith (1935). The introduction of inulin in renal function studies has been discussed by Richards, Westfall and Bott (1934), by Shannon (1934), by Professor Richards in his 1935 Harvey Lecture, and by Shannon and Smith in the above paper, and this discussion need not be extended here.

CHEMICAL METHODS. The chemical methods used in this investigation were the same as those described by Shannon and Smith (1935), except that in some of the earlier experiments sugars were determined both on a copper sulphate-sodium tungstate filtrate (Somogyi, 1931) by the Folin (1929) sugar method, as well as on the iron filtrate of Steiner, Urban and West (1932) by the Shaffer-Somogyi (1932) method. Extensive observations on recovery of inulin, glucose and xylose from plasma and urine have been made with both methods. The Folin method gives incomplete reduction with xylose but when plasma and urine are handled alike one should obtain the same U/P ratio by this as by the Shaffer-Somogyi method, especially since the saccharoid blank is low with both methods of precipitation (2 to 3 mgm. per cent with the copper and 1 to 2 mgm. per cent with the iron). Nevertheless, in experiments where both methods have been used, it has been observed that the Shaffer-Somogyi method gave values for the xylose clearance averaging five per cent lower than by the Folin method, although the inulin and glucose clearances by the two methods were identical. The reason for this discrepancy is not revealed

1 This paper is based on a thesis to be presented to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
by recoveries. All data recorded here were obtained by the Shaffer-Somogyi method on an iron filtrate. White and Monaghan (1933), using this technique, were unable to obtain the nearly exact correspondences between the creatinine, glucose and xylose clearances previously reported from this laboratory for the phlorizinized dog. We have been unable to confirm these investigators, however, and continue to secure this correspondence, except as noted above.

EXPERIMENTAL PROCEDURE. Normal female dogs weighing from 15 to 18 kgm. were used in all our experiments. A 20 per cent solution of inulin in 0.6 per cent saline, prepared at 85°C., was administered either intravenously, subcutaneously, or by both routes. One does not obtain by the subcutaneous route plasma concentrations higher than 100 mgm. per cent even when fairly large doses are given, and for this reason the inulin was given in most of our experiments intravenously in doses of from 2 to 4 grams per kilogram, or in doses of from 1 to 2 grams per kilogram subcutaneously followed by a like amount intravenously.

Xylose was administered by stomach, creatinine subcutaneously, and phlorizin intravenously or by a combination of subcutaneous and intravenous injections. The conduct of the experiments was on the whole identical with those previously reported.

RESULTS. The experiments reported in table 1 show that the rate of excretion of inulin in the dog is directly proportional to the plasma concentration between values of 53 and 565 mgm. per cent. It follows from this fact that the inulin clearance is independent of its plasma level. The curves generated by plotting plasma level against rate of excretion extrapolate through the zero coordinates, indicating that the relationship holds true at very low as well as at the observed plasma levels.

An experiment showing that the administration of inulin intravenously does not affect the clearances of urea, xylose or creatinine, either absolutely

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Showing the relationship existing between plasma level and rate of excretion of inulin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>mgm. per cent</td>
</tr>
<tr>
<td>565</td>
</tr>
<tr>
<td>380</td>
</tr>
<tr>
<td>230</td>
</tr>
<tr>
<td>143.5</td>
</tr>
<tr>
<td>86</td>
</tr>
<tr>
<td>53.5</td>
</tr>
</tbody>
</table>
TABLE 2
Data to show that the intravenous infusion of inulin has no effect on the renal clearances of other substances

<table>
<thead>
<tr>
<th>PERIOD NUMBER</th>
<th>CLEARANCE FLOW</th>
<th>PLASMA</th>
<th>CLEARANCES</th>
<th>CLEARANCE RATIOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. per min.</td>
<td>Urea</td>
<td>Xylose</td>
<td>Inulin</td>
</tr>
<tr>
<td></td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
</tr>
<tr>
<td>1</td>
<td>2.53</td>
<td>12.4</td>
<td>80.5</td>
<td>24.0</td>
</tr>
<tr>
<td>2</td>
<td>2.10</td>
<td>12.5</td>
<td>80.7</td>
<td>27.6</td>
</tr>
<tr>
<td>3*</td>
<td>1.83</td>
<td>12.6</td>
<td>81.7</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average</td>
</tr>
</tbody>
</table>

* At the conclusion of period 3, 1.5 gram per kilogram of inulin in 0.6 per cent NaCl was given by slow intravenous infusion. Five-tenths gram xylose with 20 cc. water per kilogram were given by stomach, and 0.2 gram creatinine per kilogram in water were given subcutaneously. Period 4 started 60 minutes after end of period 3.

TABLE 3
Comparison of xylose, inulin, and creatinine clearances in the normal dog
Xylose given by stomach tube, creatinine and inulin by subcutaneous injection. Dog 9, January 18, 1934.

<table>
<thead>
<tr>
<th>PERIOD NUMBER</th>
<th>CLEARANCE FLOW</th>
<th>PLASMA</th>
<th>CLEARANCE</th>
<th>CLEARANCE RATIOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. per min.</td>
<td>Xylose</td>
<td>Inulin</td>
<td>Creatinine</td>
</tr>
<tr>
<td></td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
<td>cc. per min.</td>
</tr>
<tr>
<td>1</td>
<td>3.6</td>
<td>50.3</td>
<td>104</td>
<td>13.7</td>
</tr>
<tr>
<td>2</td>
<td>3.33</td>
<td>51.6</td>
<td>106</td>
<td>14.7</td>
</tr>
<tr>
<td>3</td>
<td>3.13</td>
<td>53.1</td>
<td>108</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>2.87</td>
<td>54.0</td>
<td>109</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average</td>
</tr>
</tbody>
</table>
or relative to each other, is given in table 2. This point is an essential one in the administration of any substance for renal function studies.

The creatinine clearance is identical with that of inulin under all conditions that we have examined. A typical experiment illustrating simultaneous creatinine and inulin clearances is given in table 3, and data from the entire series are given in figure 1. The mean of 42 comparisons on 8 dogs gives a creatinine/inulin ratio of 0.994 with a standard deviation of 0.034, and maximum variations of +0.086 and −0.064. In these experi-

![Graph showing data for creatinine and inulin clearances.](https://example.com/figure1.png)

Fig. 1. Data showing that the simultaneous U/P ratios (and therefore the clear-
ances) of creatinine and inulin are equal in the normal dog. This equality is main-
tained after phlorizin.

ments the plasma creatinine varied from 6.0 to 130 mgm. per cent, and the U/P ratio of inulin from 9 to 47. Lack of space precludes the presentation of the data in full, but it may be commented that we find the creati-
ine clearance to be independent of the plasma level of this substance, as is the case with inulin.

The xylose clearance in the normal dog is less than the simultaneous inu-
lin clearance. In this finding we confirm Richards, Westfall and Bott (1934), whose data, however, show a wider deviation between the two clearances than ours do. A series of 24 observations on 6 dogs is illustrated in figure 2. The mean xylose/inulin ratio in these data is 0.734
and the standard deviation of the mean is 0.022. All these observations were made at urine flows above 0.88 cc. per minute, but with inulin U/P ratios ranging from 11.1 to 0.2. A larger series of observations on this point was not considered necessary since the creatinine clearance has been shown in a number of previous papers to exceed the xylose clearance by about this amount.

Data from a typical experiment illustrating the action of phlorizin are given in table 4, and a summary of observations of 5 dogs before and after phlorizin (30 periods in all) is given in table 5. Since phlorizin depresses all renal clearances it is necessary in making comparisons before and after the administration of this drug to take one clearance as a standard of reference. In tables 4 and 5 we have chosen the inulin clearance for this purpose. The administration of phlorizin has no effect upon the relative values of simultaneous inulin and creatinine clearances, these remaining identical within the limits of experimental error. Glucose, of course, appears in the urine after phlorizin, the clearance of this substance being identical with that of creatinine, as previously reported by Shannon, Jolliffe and Smith (1932), and therefore identical also with the inulin clearance. After phlorizin the xylose clearance rises relative to the inulin clearance, but does not come to equal it, remaining about 10 per cent below the latter. (Comparisons made by the Folin sugar method on a copper tungstate filtrate give xylose clearances about 5 per cent higher, as reported under Methods; in view of this fact the xylose clearance may really be within 5 per cent of the inulin clearance in the phlorizinized ani-

![Fig. 2. Data showing that the U/P ratio (and therefore the clearances) of xylose in the normal dog is less than the simultaneous U/P ratio of inulin. The dotted line represents a xylose/inulin ratio of 1.0, and the solid line a ratio of 0.734, the mean of the observations. Phlorizin raises the xylose clearance to within 10 per cent of the inulin clearance.](http://ajplegacy.physiology.org/Downloadedfrom)
TABLE 4
Comparison of urea, xylene, glucose, inulin and creatinine clearances before and after phlorizin (100 mgm. per kilogram intravenously)


<table>
<thead>
<tr>
<th>PERIOD NUMBER</th>
<th>URETHRAL FLOW</th>
<th>PLAasma CLEARANCE</th>
<th>CLEARANCE RATIOs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. per min.</td>
<td>mgm. per cent</td>
<td>cc. per min.</td>
</tr>
<tr>
<td>1</td>
<td>2.30</td>
<td>11.5</td>
<td>75.0</td>
</tr>
<tr>
<td>2</td>
<td>1.65</td>
<td>11.6</td>
<td>73.4</td>
</tr>
<tr>
<td>3</td>
<td>1.20</td>
<td>11.7</td>
<td>66.8</td>
</tr>
</tbody>
</table>

Average ..................................................... 0.53 0.72 1.00

* Following Phlorizin, 100 mgm. per kilogram, intravenously.

TABLE 5
Summary of comparisons of urea, xylene, inulin, creatinine and glucose clearances before and after phlorizin

BEFORE PHLORIZIN

<table>
<thead>
<tr>
<th>ANIMAL NUMBER</th>
<th>Number of periods</th>
<th>Clearance ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea</td>
<td>Xylene</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>35.5</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>46.6</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>72.2</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>37.3</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>75.7</td>
</tr>
</tbody>
</table>

Average ..................................................... 0.58 0.75 1.00

AVERAGE OF ALL PERIODS.

AVERAGE OF ALL PERIODS.

* Average of all periods.
EXCRETION OF INULIN BY THE DOG

In a limited series of experiments no significant change has been observed in the urea/inulin ratio before and after phlorizin.

The excretion of sucrose relative to inulin has not been extensively examined. In those experiments where this substance was present the results were in accord with the relationship reported here between xylose and inulin.

Discussion. The only independent evidence that can be advanced that inulin is not excreted in part by tubular secretion in the normal dog, is the fact that its clearance is independent of the concentration in the plasma, between wide limits of the latter. Since this evidence is not absolutely exclusive, the presumption against secretion must rest upon the evidence obtained from the glomerular fish (Richards, Westfall and Bott, 1934; Shannon, 1934) and the behavior of carbohydrates in general, as discussed by Shannon and Smith (1933). It is significant that in the dog the creatinine clearance is independent of the plasma level of this substance, as has previously been pointed out from this laboratory, and confirmed in the experiments described in this paper. The behavior of this substance in the dog is quite different from what it is in man, where a markedly curvilinear relationship is observed (Shannon, 1935). Granted that a linear relationship between plasma level and rate of excretion is, in these two instances, evidence against secretion of either substance, it is a confirmation of this evidence that the simultaneous inulin and creatinine clearances are equal, within the experimental error of observation, in both the normal and phlorizinized dog.

It follows from the above interpretation regarding the non-secretion of inulin that some xylose is reabsorbed in the normal dog, the figures presented here indicating the fraction to be about 27 per cent of that which is filtered. The fact that the xylose/inulin ratio does not vary with variation in inulin U/P from 11.1 to 76.2 might be taken to indicate that the degree of concentration of the urine is not the predominating factor in the discrepancy between the excretion rates of these two substances.

Since phlorizin raises the xylose clearance relative to the inulin clearance, it must be inferred that reabsorption in the dog, as in the dogfish and man, is in part an active process. In the three experiments on the phlorizinized dog reported here, the xylose clearance is still 10 per cent below the inulin clearance, a difference that is only 5 per cent when the Folin method, instead of the Shaffer-Somogyi method, is used. In view of the much larger series of comparisons previously published from this laboratory (in which the Folin sugar method and copper tungstate filtrate were used), showing almost exact agreement in the clearances of these substances in the phlorizinized animal, the significance of this difference is questionable.

Shannon, Jolliffe and Smith (1932) based their conclusion that creati-
nine was secreted in the normal dog on the facts that: a, the creatinine clearance exceeds the simultaneous xylose and sucrose clearances; b, under phlorizin, these clearances come to equal each other, an equalization that is effected by a fall in creatinine clearance rather than a rise in xylose clearance; and c, phlorizin does not change the urea/xylose ratio, as might be expected if the drug were specifically blocking the reabsorption of the pentose. Pitts (1934) in a subsequent examination of the excretion of creatine, confirmed the first two observations, noting that on the administration of phlorizin, the creatine and creatinine clearances dropped to approximately the same level, the glucose rose to the xylose level, and the xylose clearance itself was not appreciably changed. The constancy of the xylose clearance before and after phlorizin may, however, be an artifact due to a decrease in glomerular filtration equal to a diminished reabsorption of the sugar. The fact that in Pitts' (1934) experiments the average creatine/creatinine ratio remained constant (0.80 before and 0.92 after phlorizin) in spite of a fall in the absolute value of both clearances, whereas the ratio of the xylose clearance to the other two rose, points in this direction. The apparent constancy of the urea/xylose ratio before and after phlorizin, which Jolliffe, Shannon and Smith (1932) emphasized as evidence against reabsorption of xylose, has also been noted in man (Chassis, Jolliffe and Smith, 1933) but, as Shannon and Smith (1935) have pointed out, this ratio is now known to change with changes in the absolute level of these clearances, and such independent changes may partly or wholly occlude the change due to the blockage of the reabsorption of the sugar.

It would appear from the evidence obtained by the use of insulin that xylose is actively reabsorbed in the normal dog, and that the difference between the xylose and creatinine clearances is due to this reabsorption. The extent of this reabsorption, as judged from the inulin clearance, appears to be about the same in the dog as in the dogfish and man.

When we consider all the evidence available on the dogfish, man, and dog, we are led to the following interpretation: that inulin is not secreted by any of these and, assuming no reabsorption of this substance (*vide infra*), its clearance is very close to, if not identical with, the rate of glomerular infiltration. The capacity to secrete creatinine, evident in the dogfish and man, is vestigial or absent in the dog; hence the equality of the inulin and creatinine clearances. This equality is not perturbed by phlorizin, and since phlorizin brings the glucose clearance up to the inulin and creatinine clearances, it appears that this drug completely blocks the reabsorption of glucose in the renal tubules, as was believed by Jolliffe, Shannon and Smith (1932).

Since an apparent active reabsorption of some xylose has been uncovered in these experiments, it seems possible that there might also be an active reabsorption of some inulin. No evidence bearing on this question can be
advanced at the present time, unless it be the mere fact of the identity of
the creatinine and inulin clearances of the normal animal.

With regard to the passive reabsorption (diffusion) of inulin, it will be
noted that the equality between the creatinine and inulin clearances holds
at U/P ratios of inulin varying from 9 to 17, and that in the phlorizinized
dog this equality also obtains with the simultaneous glucose clearance. It
has been shown that the simultaneous xylose and sucrose clearances in
the normal dog, and xylose and glucose clearances in the phlorizinized dog,
are equal (Jolliffe, Shannon and Smith, 1932; Pitts, 1934); it appears from
these facts that the differential diffusion of these substances must be very
small, and that the diffusion of a molecule as large as inulin (mol. wgt. 972
or greater) is probably negligible. But this argument cannot be extended
to the abnormal kidney, or to the normal kidney at very low urine flows,
without the support of further evidence.

SUMMARY

The rate of inulin excretion in the dog is directly proportional to plasma
concentration between values of 53 and 565 mgm. per cent.

The intravenous administration of inulin does not affect the urea, xylose
or creatinine clearances.

In a series of 42 comparisons on 8 dogs, the ratio of the simultaneous
creatinine and inulin clearances has a mean value of 0.994 with a standard
deviation of 0.034, and maximum variations of +0.086 and -0.064.

The xylose clearance in the normal dog at moderate to high urine flows
is less than the simultaneous inulin clearance, the mean xylose/inulin
ratio being 0.734, with a standard deviation of 0.022.

Under phlorizin the glucose clearance rises to the level of the creatinine
and inulin clearances, the equality of which is maintained, while the xylose
clearance is raised to within 10 per cent of the inulin clearance.

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